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NIXON & VANDERHYE, PC
901 NORTH GLEBE ROAD, 11TH FLOOR
ARLINGTON, VA 22203

EXAMINER	
MITCHELL, LAURA MCGILLEM	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/501,777	BIRCH ET AL.	
	Examiner	Art Unit	
	Laura M. Mitchell	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *25 October 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1, 3-7, 10-11, 14-15, 17-24, 27-31* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-7,10,11,14,15,17-24 and 27-31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/25/2007 has been entered.

It is noted that claims 23 and 27 have been amended, claims 2, 8-9, 12-13, 16, 25-26 are cancelled and claims 14, 17, 21 and 28-31 have been added. Claims 1, 3-7, 10-11, 14-15, 17-24 and 27-31 are under examination.

Claim Rejections - 35 USC § 112

Claims 14 and 23 have been amended to change their dependency from cancelled claim 13 to claim 11, drawn to a process. Claims 17 and 21 have been amended to change their dependency from cancelled claim 16 to claim 1, drawn to a cell. The rejection of claims 14-15, 17-18, and 21-24 under 35 U.S.C. 112, second paragraph, as being indefinite has been withdrawn.

Double Patenting

Claims 25-26 have been canceled, therefore they are longer substantial duplicates of claims 19-20. The provisional objection of claims 25-26 is moot.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 3-7, 10-11, 19-24 and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bebbington et al (U.S. Patent No. 5,891,693, of record) as evidenced by Barsomian et al (U.S. Patent No, 5,238,821, of record) in view of Brandt et al (U.S. Patent No. 6,395,484, of record). It is noted that previously rejected claims 25-26 have been cancelled. Amended claims 21-24 and new claims 28-29 are newly added to this rejection (below arguments and response to arguments). **This rejection is being maintained for reasons of record in the previous Office Action, mailed 7/25/2007 and for reasons outlined below**.

Applicants request clarification in the event the rejection over the combination of cited art is maintained as the applicants believe that the cells as described in Bebbington et al and when compared to the cells as claimed in the present application have only the presence of GS and the expression of a heterologous proteins in common.

The Applicants therefore consider Brandt et al as being the closest prior art, which would not have made the claimed invention obvious. Specifically, as outlined above, Brandt et al discloses the use of human glutamine auxotrophic cells such as HT1080; the use of serum free medium; the use of a selection /amplification marker

such as DHFR to produce glycosylated proteins (such as EPO) comprising sialic acid moieties. Applicants submit that it should however be noted that Brandt et al does not actually show glycosylation or the presence of sialic acid moieties. Thus, Applicants submit that a difference between Brandt et al and the claimed invention is the presence of the additional selection marker GS in the cell.

Applicants submit that the effect of the presence of GS in said human glutamine auxotrophic cell - according to the claimed invention - is not only the ability of the cell to grow in glutamine free medium, but most importantly is the cell's ability to exhibit elevated specific rates of protein synthesis, improved protein activity, enhancement of the protein quality (such as enhanced sialylation) and extended cell viability. Applicants submit that these additional effects of GS were clearly surprising and have not been anticipated or rendered obvious by any of the cited prior art documents including Bebbinqton et al.

Applicants submit that in detail: Examples 6, 8 and 11 of the present application show that the HT1080 human cell line transfected with EPO and DHFR gene (named R223; Example 1 - R223 which is used as starting point for the present invention is thus similar to the cell line as described by Brandt et al) and supertransfected with GS exhibited elevated specific rates of EPO synthesis compared to the R223 cell line (Table 5 - growth of cells in medium with serum; and Table 7 and 12 - growth of cells in serum-free suspension culture).

Further, Applicants submit that examples 9 and 11 of the present application show that the EPO produced by the GS supertransfected cell exhibited intensification of

the more acidic isoforms when compared to the EPO produced by the R223 cell line, which indicates an increased degree of sialylation of EPO produced by the GS supertransfectant cell line (page 27, line 4 to 5 of WO 03/054172) - while the more acidic bands possess the highest biological activity (page 28, line 1). Still further, Applicants submit that Example 10 of the present application shows that the cell line supertransfected with GS has extended cell viability and thus, an increase in duration of culture resulting in an increase in product concentration (Table 11).

Applicants submit that none of these effects of GS have described or remotely suggested by Bebbington et al or Barsomian et al. Applicants submit that the person ordinarily skilled in the art would not have envisaged to transfect the glutamine-auxotropic human cell stably expressing EPO with the help of the DHFR selection marker - as, for example, disclosed in Brandt et al. - with the additional GS marker - the GS marker only being disclosed for example in Bebbington et al. - to produce a product with improved cell viability and product quality such as glycosylation.

For completeness, the Applicants note the Examiner's comment on page 11 of the Office Action dated July 25, 2007, that the claims do not reflect the requirements of supertransfection with GS or of enhanced sialylation. The applicants note in response that although the term "supertransfection with GS" is not explicitly mentioned in the claim, the presence of GS in addition to another selection marker such as DHFR is clearly a feature of the claims. Applicants submit that new claims 28 to 31 further emphasize the step of "supertransfection", i.e. the additional transfection of GS selection marker gene. As regards to the enhanced sialylation, the applicants note that

claims 1 and 7 require a "sialylated" protein, and that the enhancement of sialylation is a direct consequence of using the claimed cell or process according to the claims.

Applicant's arguments filed 10/25/2007 have been fully considered but they are not persuasive. Applicants' first point submits that Brandt et al does not actually show glycosylation or the presence of sialic acid moieties. It is noted that claims 1-6 and 21-22 are drawn to the glutamine-auxotrophic cell itself, wherein it comprises an exogenous DNA sequence encoding a sialylated protein or an exogenous DNA sequence capable of altering the expression of an endogenous gene encoding a sialylated protein and an exogenous sequence encoding a glutamine synthase. Although a limitation of the cell as claimed is that it must be capable of producing the sialylated protein and capable of growing in a glutamine free and serum free medium, in order to meet the limitation of the claim the cell does not have to actually express the sialylated protein, it must only be capable of expression of the sialylated protein. As long as the recited DNA sequences are present in the cell, it would meet the structural limitations recited in the claims.

However, as discussed in the previous Office Action (page 5), Bebbington et al contemplate embodiments wherein the heterologous protein can be tissue plasminogen activator (tPA) (see column 2, lines 31-36, for example). Barsomian et al disclose that tPA is a sialylated glycoprotein (see column 2, lines 49-52, for example). Brandt et al also contemplate embodiments of cells for the production of proteins such as tPA (see column 3, lines 61-65) and disclose that the cells as disclosed synthesize a protein with a correct glycosylation pattern, especially with regard to the sialic acid residues (see

column 3, lines 22-46, for example). Absent evidence to the contrary, if a cell as claimed were transfected with an expression vector comprising the sequence encoding tPA, the tPA would be sialylated to at least some degree. Bebbington et al also teach embodiments of vectors that comprise sequences encoding xanthine-guanine phosphoribosyl transferase (see column 5, lines 45-62, for example). It is also noted that Brandt et al does not have to show the presence of sialic acid molecules in an example because if the limitation is contemplated in the specification. Therefore, Bebbington et al as evidenced by Barsomian in view of Brandt et al render obvious a cell with the limitations recited in claim 1.

Although Applicants submit that a difference between Brandt et al and the claimed invention is the presence of the additional selection marker GS in the cell, it is not necessary for Brandt et al to teach this limitation because it is taught by the primary prior art (Bebbington et al) reference. Bebbington et al teach mouse and rat lymphoid cell lines that can be transformed to glutamine independence by incorporating a gene encoding GS so that the cells can grow in glutamine-free medium.

Applicants submit that the effect of the presence of GS in said human glutamine auxotrophic cell is most importantly is the cell's ability to exhibit elevated specific rates of protein synthesis, improved protein activity, enhancement of the protein quality (such as enhanced sialylation) and extended cell viability. However, as claim 1 is written, there is no functional limitation for the glutamine synthetase, and since Bebbington et al teach that the purpose of including a sequence encoding GS is to allow the cells to grow in glutamine free medium, this is sufficient reason to include the GS sequence. The

cell's ability to exhibit qualities such as enhanced sialylation and protein quality, etc. as an effect of the exogenous GS are not limitations of the cell product claims.

The motivation provided in the previous Office action to combine the teachings of Bebbington et al and Brandt et al to use a human glutamine auxotrophic cell that is transfected with a glutamine synthetase sequence and a separate vector comprising a sequence for an exogenous sialylated protein is the expected benefit of being able to synthesize a target protein with a sialic acid moiety glycosylation pattern that is comparable to that of the naturally occurring target protein (see column 3, lines 23-30, for example) is sufficient motivation to combine and render obvious the cell comprising the recited structural limitations. Although Applicants submit that these additional effects of GS were clearly surprising, the effects of the GS are not recited limitations of the cell product claims. The motivation to combine the teachings of Bebbington et al and Brandt et al does not have to be the expectation of producing those surprising effects of GS on protein synthesis activity and quality when another motivation is provided.

Although Applicants submit that examples 9 and 11 exemplify GS supertransfectant cell line-based production of a protein with an increased degree of sialylation of EPO, the degree of sialylation is not a limitation of the claims drawn to the glutamine –auxotrophic cell, only that the cell would comprise a DNA sequence encoding the sialylated protein (i.e. claims 1, 2 and 19-20).

While Applicants submit that Example 10 of the present application shows that the cell line supertransfected with GS has extended cell viability and thus, an increase in duration of culture resulting in an increase in product concentration, the viability and

culture duration of the cell as claimed is not a limitation of the claimed cell and therefore it is not necessary for the prior art references to teach these effects. As discussed previously, the motivation to combine the teaching to produce a glutamine –auxotrophic human cells with an additional GS marker is the expected benefit of being able to synthesize a target protein with a sialic acid moiety glycosylation pattern that is comparable to that of the naturally occurring target protein (see column 3, lines 23-30, for example) and does not have to be the benefit of producing a protein product with improved quality and increase cell viability. Therefore Bebbington et al as evidenced by Barsomian et al in view of Brandt et al render obvious a human cell comprising an exogenous sequence encoding a sialylated protein (e.g. tPA) further comprising a selectable marker (e.g. DHFR) and an exogenous GS sequence wherein these sequences are on more than one DNA vector and the cell is capable of producing the protein and capable of growing in a glutamine-free and serum-free medium (**claim 1**). Bebbington et al as evidenced by Barsomian et al in view of Brandt et al also render obvious claims 3-6 as specifically discussed in the previous Office action pages 8-9.

Rejected claims 7, 10-11 and 19-20 are drawn to a process for producing a sialylated protein using the cell of claim 1. In claim 7, DNA sequence encoding a sialylated protein is expressed and recovered. Bebbington et al teach an embodiment of host cells comprising vectors comprising a sequence encoding EPO to be expressed as protein (see column 9, lines 10-17, Example 4, and Example 5, column 10, lines 14-20, in particular), which meets the limitation of a glutamine-auxotropic human cell comprising a DNA sequence encoding a sialylated protein, wherein the sialylated

protein is EPO. Brandt et al also contemplate embodiments for use of cells for the production of proteins such as EPO and disclose that the cells as disclosed synthesize a protein with a correct glycosylation pattern especially with regard to the sialic acid residues (see column 3, lines 22-46, for example). Brandt et al teach that the cells were cultured to produce the target protein, which was then recovered and quantified from the cell supernatant (see column 6, lines 44-63, for example). Brandt et al also illustrates data regarding transient EPO glycosylation (see column 8, Table 1, in particular). Therefore in contrast to Applicants submission that Brandt et al do not actually show glycosylation or the presence of sialic acid residues, Brandt et al disclose, exemplify and contemplate embodiments of a method to produce a sialylated protein.

As written, claims 7, 10-11 and 19-20, drawn to a process for producing a sialylated protein, do not recite limitations regarding rate of protein synthesis, protein activity, enhanced quality or sialylation extended host cell viability. Applicants submit that these effect were surprising and therefore would not be anticipated or rendered obvious by the cited references. However, since these effects are not limitations of the claims it is not necessary for the applied prior art references to anticipate or suggest such effects in order to render obvious the claimed process because the references render obvious the limitations that are recited.

Although Applicants submit that Ex. 9 and Ex. 11 exemplify GS supertransfectant cell line-based production of a protein with an increased degree of sialylation of EPO, an increased degree of sialylation is not a limitation of the claims drawn to the process

of producing a sialylated protein from a glutamine –auxotrophic cell, only that produced and recovered protein would be sialylated (i.e. claims 7; 10-11 and 19-20).

While Applicants submit that Example 10 of the present application shows that the cell line supertransfected with GS has extended cell viability and an increase in duration of culture resulting in an increase in product concentration, the viability and culture duration of the cell as claimed is not a limitation of claims drawn to the process of producing a sialylated protein from a glutamine-auxotrophic cell. Therefore it is not necessary for the prior art references to suggest or render obvious these effects.

As discussed previously, the motivation to combine the teaching to produce a sialylated protein in glutamine –auxotrophic human cells with an additional GS marker is the expected benefit of being able to synthesize a target protein with a sialic acid moiety glycosylation pattern that is comparable to that of the naturally occurring target protein (see column 3, lines 23-30, for example) and does not have to be the benefit of producing a protein product with improved quality and increase cell viability. Therefore Bebbington et al as evidenced by Barsomian et al in view of Brandt et al do render obvious the process as claimed in claim 7 for example.

Bebbington et al teach an embodiment of a method and a host cell comprising vectors comprising a sequence encoding EPO to be expressed as protein (see column 9, lines 10-17, Example 4, and Example 5, column 10, lines 14-20, in particular), which meets the limitation of a method and glutamine-auxotropic human cell comprising a DNA sequence encoding a sialylated protein, wherein the sialylated protein is EPO (**claims 21 and 23**).

Bebbington et al teach that the DNA sequence encoding EPO is a human EPO gene sequence (see column 9, lines 19-25, in particular), which meets the limitation of a method and glutamine-auxotropic human cell comprising a DNA sequence encoding a sialylated protein, wherein the sialylated protein human EPO (**claims 22 and 24**).

New claim 28 is drawn to a method to produce the glutamine-auxotropic human cell of claim 1 or 17 comprising the steps of transfecting said human cell with an exogenous DNA sequence encoding a protein or an exogenous DNA sequence capable of altering the expression of an endogenous gene encoding a protein, and which exogenous DNA sequence further comprises a selectable marker selected from the group consisting of DHFR, adenosine deaminase, asparagine synthetase, aspartate transcarbamylase, metallothionein-1, ornithine decarboxylase, P-glycoprotein, ribonucleotide reductase, thymidine kinase and xanthine-guanine phosphoribosyl transferase and (b) transfecting the cell obtained in step (a) with an exogenous DNA sequence encoding a glutamine synthetase, and (c) culturing the cell obtained in step (b) in glutamine-free medium. Dependent claim 29 is drawn to the method wherein the cell is adapted to growth in serum free medium.

Bebbington et al teach an embodiment of a vector comprising a gene for GS used for transforming a cell line to glutamine independence (see column 3, lines 7-15, for example). Bebbington et al teach examples of heterologous proteins as human growth hormone, or tPA or tissue inhibitor of metalloproteinase (see column 2, lines 31-36, for example). Bebbington et al also teach embodiments of vectors that comprise

sequences encoding xanthine-guanine phosphoribosyl transferase (see column 5, lines 45-62, for example). Bebbington et al teach a method in which host cell lines are cotransformed with a vector comprising the sequence of a GS gene and a vector comprising a heterologous protein and/or selectable marker (see column 2, lines 7-14, for example).

Bebbington et al teach growth of transformed host cells in glutamine free DMX medium (see column 7, lines 1-10, for example). Bebbington et al do not teach a human glutamine-auxotrophic cell. Bebbington et al do not teach an exogenous sequence encoding a sialylated protein that further comprises a selectable marker such as claimed. **Bebbington et al do not teach growing the cells in serum-free medium.**

Brandt et al teach human HT1080 cells for the preparation of human proteins in order to produce human proteins in economical yields in a form that is suitable for production of a pharmaceutical preparation (see column 1, lines 12-17, for example) which meets the limitation of glutamine-auxotropic human cell line. Brandt et al teach that it is advantageous to use a human cell line that synthesizes a desired protein with a glycosylation protein, especially a sialic acid protein comparable to that of the naturally occurring target protein (see column 3, lines (19030, for example). Brandt et al also teach that a negative or positive selection marker or amplification gene can be included and can be DHFR, adenosine deaminase, ornithine decarboxylase or a thymidine kinase gene (see column 4, lines 56-65, for example), which meets the limitation of a gene further comprising a selectable marker selected from the group recited in claim 28.

Brandt et al teach the advantage of using serum free culture medium for culture of human cells because purification of proteins from serum free culture is substantially easier and has no danger of contamination with animal pathogens (see column 2, lines 22-34, for example).

It would have been obvious to the skilled artisan at the time the invention was made to modify method taught by Bebbington et al from making a rodent glutamine auxotrophic cell to produce an exogenous sialylated protein such as tPA to a human cell as taught by Brandt et al to produce a human exogenous sialylated protein because Brandt et al disclose that human cells are preferred for the preparation of human proteins in order to produce human proteins in economical yields in a form that is suitable for production of a pharmaceutical preparation. The motivation to make a human glutamine auxotrophic cell that is transfected with a glutamine synthetase sequence and a separate vector comprising a sequence for an exogenous sialylated protein is the expected benefit of being able to use the cells to synthesize a target protein with a sialic acid moiety glycosylation pattern that is comparable to that of the naturally occurring target protein (see column 3, lines 23-30, for example).

It also would have been obvious to the skilled artisan at the time the invention was made to modify the method and cell taught by Bebbington et al and incorporate a second exogenous DNA sequence as a selection marker and amplification gene because Brandt et al teaches clone selection and gene amplification using a positive or negative selection marker. The motivation to use a selection marker and amplification gene such as DHFR to make a cell is the expected benefit as disclosed by Brandt et al

of being able to use a gene with a sensitivity for a selection agent in order to increase the expression of an gene to be produced by culturing the cell in the presence of increasing concentrations of a selection agent (i.e. methotrexate) (see column 10, lines 36-44, for example). There is a reasonable expectation of success to make a human glutamine auxotrophic cell wherein these exogenous DNA sequences are located on more than one DNA vector construct, because similar methods have worked previously in the references cited. Claim 28 appears to recite limitations regarding the order of the vector transformations. Limitations of this type would amount to routine optimization of the method. Therefore, Bebbington et al in view of Brandt et al render obvious a method to produce the glutamine-auxotropic human cell of claim 1 as claimed in **claim 28**.

It also would have been obvious to the skilled artisan at the time the invention was made to modify the method taught by Bebbington et al and make a glutamine auxotrophic cell that is capable of growing in serum-free medium because Brandt teach the importance of serum-free medium for cultivation. The motivation to use a serum-free medium is the expected benefit of being able to reduce the danger of contamination of the protein produced with animal pathogens that might be introduced by using animal serum (see column 2, lines 22-35, for example). There is a reasonable expectation of success to make a human glutamine auxotrophic cell with these limitations, because similar method have worked previously in the references cited. Therefore Bebbington et al as evidenced by Barsomian et al in view of Brandt et al render obvious a method to produce the glutamine-auxotropic human cell of claim 1 wherein the cell is further adapted to growth in serum free medium as claimed in **claim 29**.

Applicants appear to have added claim 28-31 in an effort to encompass supertransfection (i.e. the additional transfection of GS selection marker gene) with GS or of enhanced sialylation. The applicants note that the presence of GS in addition to another selection marker such as DHFR is clearly a feature of the claims. As regards to the enhanced sialylation, the applicants note that claims 1 and 7 require a "sialylated" protein, and that the enhancement of sialylation is a direct consequence of using the claimed cell or process according to the claims. However as discussed above, in order to meet the limitation of the cell as claimed, or the process of using the cell to make the sialylated protein, or the process of making the cells, the prior art references are not required to suggest or render obvious specific levels of sialylation or changes in degree of sialylation because these aspects are not limitations recited in the claims. The structural limitations of the host cells and the steps of the claimed processes can be rendered obvious by the cited prior art reference without disclosure of the effects as submitted by the Applicants. It is also noted that the method of claims 27-31 do not recite the limitation that the exogenous DNA encoding protein should encode a protein that is a sialylated protein, and therefore encompass a method wherein the protein is non-sialylated or glycosylated. The instant specification discloses embodiments of proteins to be expressed that are non-glycosylated. (see paragraph 0033). Therefore it would not be necessary for the cell produced by the methods of claim 28-31 to make any protein with specific levels of sialylation or changes in degree of sialylation to meet the recited claim limitations.

Claims 30-31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bebbington et al (of record) in view of Brandt et al (of record) and further in view of Meyers et al (U.S. Patent No. 6,110,663, 8/29/2000).

Applicants claim a method to produce the glutamine-auxotropic human cell claim 1 or 17 comprising the steps of (a) transfecting a cell which is stably transfected with an exogenous DNA sequence encoding a protein or an exogenous DNA sequence capable of altering the expression of an endogenous gene encoding a protein, and which exogenous DNA sequence further comprises a selectable marker selected from the group consisting of DHFR, adenosine deaminase, asparagine synthetase, aspartate transcarbamylase, metallothionein-1, ornithine decarboxylase, P-glycoprotein, ribonucleotide reductase, thymidine kinase and xanthine-guanine phosphoribosyl transferase, with a further exogenous DNA sequence encoding a glutamine synthetase, (b) culturing the cell obtained in step (a) in glutamine-free medium.

The teachings of Bebbington et al and Brandt et al have been discussed in detail in the previous Office action and above. As demonstrated above, Bebbington et al in view of Brandt et al render obvious a method to produce the glutamine-auxotropic human cell of claim 1 comprising transfecting the cell with an exogenous DNA sequence encoding protein and a selectable marker and also transfecting the cell with exogenous DNA sequences encoding a glutamine synthetase and culturing the cell in glutamine free medium. **Bebbington et al and Brandt et al do not teach the stable transfection of the cell with an exogenous DNA sequence encoding protein and a selectable marker.**

Meyers et al teach cells comprising expression vectors that include reporter genes used in cell assays (see abstract, column 3, lines 4-17, for example). Meyers et al teach that the reporter gene can include any protein that can be detected by antibodies using immunohistological staining or FACS methods (see column 5, lines 9-25, for example). Meyers et al teach transfection of human cell line with vectors for reporter assays. Meyers et al disclose that the cells can be either transiently or stably transfected. Meyers et al teach that it is advantageous to use stable transfection to ensure that essentially every cell in the population contains the desired reporter construct and that times for cell assays can be investigated in intervals from hours to days (see column 6, lines 21-38, in particular).

It would have been obvious to the skilled artisan at the time the invention as made to modify the method rendered obvious by Bebbington et al in view of Brandt et al and stably transfect a glutamine-auxotrophic human cell with an exogenous DNA sequence encoding a protein such as tPA or EPO and then transfect with a DNA sequence comprising a GS gene because Meyers et al teach that it is advantageous to use stable transfection for cell comprising sequences for recombinant genes. The motivation to stably transfect the cell with one of the DNA constructs is the expected benefit taught by Meyers of being able to ensure that essentially every glutamine-auxotrophic human cell in the population contains the desired construct. There is a reasonable expectation of success to be able to produce the cell as claimed in claim 30 by stably transfecting the cell with an exogenous DNA sequence encoding a protein such as tPA or EPO because methods of stable transfection are well known in the prior

art and similar methods have worked in the Meyers et al reference. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore Bebbington et al in view of Brandt et al and further in view of Meyers render obvious the method of **claim 30**.

It also would have been obvious to the skilled artisan at the time the invention was made to modify the method rendered obvious by Bebbington et al in view of Brandt et al for making a glutamine auxotrophic cell that is capable of growing in serum-free medium because Brandt teach the importance of serum-free medium for cultivation. The motivation to use a serum-free medium is the expected benefit of being able to reduce the danger of contamination of the protein produced with animal pathogens that might be introduced by using animal serum (see column 2, lines 22-35, for example). There is a reasonable expectation of success to make a human glutamine auxotrophic cell with these limitations, because similar method have worked previously in the references cited. Therefore, Bebbington et al in view of Brandt et al render obvious a method to produce the glutamine-auxotropic human cell of claim 1 wherein the cell is further adapted to growth in serum free medium as claimed in **claim 31**.

Claims 14-15 and 17-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bebbington et al (of record) as evidenced by Barsomian (of

record) in view of Brandt et al (of record) and further in view of Hermentin et al (U.S. Patent No. 6,096,555, of record).

Applicants claim a cell and a process for the production of a protein comprising culturing a glutamine-auxotropic human cell in glutamine free medium and recovering the expressed sialylated protein comprising tri-, tetra- or pentasialo glycoforms. Applicants also claim a cell and a process wherein the sialylation is defined by an N-glycan charge.

The teachings of Bebbington et al as evidenced by Barsomian, and the teaching of Brandt et al have been detailed above and in previous Office actions.

Specific to this rejection, Brandt et al teach that the target protein EPO is glycosylated (see page 10, paragraph 1.7, for example). Brandt et al teach that it is desirable to have a human EPO protein produced with a comparable glycosylation pattern, especially in regard to sialic acid residues.

As discussed previously, Bebbington et al as evidenced by Barsomian et al in view of Brandt et al render obvious a human cell comprising an exogenous sequence encoding a sialylated protein further comprising a selectable marker (e.g. DHFR) and an exogenous GS sequence wherein these sequences are on more than one DNA vector and the cell is capable of producing the protein and capable of growing in a glutamine-free and serum-free medium. Bebbington et al as evidenced by Barsomian et al in view of Brandt et al also render obvious a process for producing a sialylated protein using the glutamine auxotrophic human cell wherein the cell is cultured in medium that is both serum free and glutamine free. **Brandt et al and Bebbington et al do not teach that**

sialylation is defined by N-glycan charge or that the sialylated protein comprises tri-, tetra- or pentasialo glycoforms of N-glycan.

Hermentin et al teach a process for characterizing the glycosylation of glycoproteins based on a hypothetical charge number N. Hermentin et al teach that it is important to reliably determine the degree of glycosylation or sialylation in glycoproteins, such as erythropoietin, in order to gauge bioavailability/biological activity of a protein for therapeutic use. Hermentin et al discloses that when erythropoietin is incompletely glycosylated, it is quickly cleared from the blood circulation and would not be biologically useful (see column 1, lines 6-15, 28-45 and column 2, lines 14-25, for example). Hermentin et al teach that it is crucial to determine the distribution of glycan groups exhibiting differing degrees of sialylation to be able to index the bioavailability of a glycoprotein. Hermentin et al teach that the N charge of a glycoprotein is determined in part by determining the percentage of trisialo, tetrasialo and pentasialo ranges (see column 3, lines 27-50, column 4, lines 27-35, column 5, lines 4-12, for example). Hermentin et al teach that the N-glycan charge value was determined for EPO and erythropoietin is comprised of trisialylated N-glycans and tetrasialylated glycans (see column 12, lines 43-52, for example), which meets the limitation of EPO as a sialylated protein comprising tri and tetrasialylated glycoforms defined by N-glycan charge and a process of defining sialylation by N-glycan charge.

It would have been obvious to the skilled artisan at the time the invention was made to determine the N-glycan charge for the sialylated protein such as EPO or tPA being produced by the cell because Hermentin et al teach that it is important to know

the degree of glycosylation of recombinant therapeutic proteins such as erythropoietin, since slightly altered glycosylation patterns can drastically effect the activity of the therapeutic protein. The motivation to determine the N-glycan charge is the expected benefit of being able to determine the degree of glycosylation in a simple, reliable manner suitable for replacing the methods previously known in the art for determining the bioavailability of a therapeutic protein before use. There is reasonable expectation of success in combining the protein production method using a glutamine auxotrophic cell rendered obvious by Bebbington et al in view of Brandt et al with the methods taught by Hermentin et al and use a glutamine- auxotropic human cell transfected with an exogenous DNA sequence encoding a glycoprotein, such as erythropoietin, to produce and recover erythropoietin and determine its bioavailability via N-glycan charge, because these methods have worked before in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore, Bebbington et al as evidenced by Barsomian in view of Brandt et al and further in view of Hermentin et al render obvious a cell and a process for the production of a sialylated protein comprising culturing a glutamine-auxotropic human cell in glutamine free medium and recovering the expressed sialylated protein comprising tri-, or tetrasialo glycoforms of the glycan defined by an N-glycan charge (claims 14-15 and 17-18).

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura M. Mitchell whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Laura McGillem Mitchell
Examiner
1/17/2008

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